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Varied effect of fortification of kale sprouts with novel organic selenium compounds on the synthesis of sulphur and phenolic compounds in relation to cytotoxic, antioxidant and anti-inflammatory activity

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ABSTRACT

Selenium deficiency in daily diet is a common problem in many countries, thus searching for new dietary sources of this trace element is an important scientific challenge. Selenium biofortified sprouts from Brassicaceae family are good candidates for new dietary selenium source, as they reveal one of the highest capability to synthesize and accumulate this element. As a part of this extensive search, the influence of novel selenium organic compounds on fortification of kale sprouts biological activity was investigated. The present study is focused on the evaluation of the influence of these compounds on the synthesis of glucosinolates, isothiocyanates, indoles and phenolic acids in kale sprouts, together with the determination of their impact on antioxidant, anti-inflammatory and cytotoxic activity on gastrointestinal, prostate, and thyroid normal and cancer cells. The present study yields the conclusion that fortification of kale sprouts with selenium organic compounds bearing benzoselenoate scaffold influences the production of isothiocyanates, phenolic acids, and enhances the antioxidant properties of fortified sprouts. Notably, fortification with compounds based on benzoselenoate scaffold display chemoprotective properties in various cancer types (gastric, thyroid, and prostate cancer). The present study can facilitate the design of future agrochemicals. Compounds bearing benzoselenoate scaffold or selenyl phenylpiperazine motif seem to be particularly promising for these purposes.

1. Introduction

Selenium is one of the trace elements of great importance for human health, responsible for a number of physiological functions in the organism. An optimal selenium supplementation decreases the risk of noncommunicable diseases, including cancer [1]. As selenium deficiency in daily diet is a common problem in many countries, searching for new dietary sources of this element is an important scientific challenge. To produce selenium biofortified food, especially vegetables, sprouts and plant based functional foods may be a valuable idea to solve this problem, providing preventive effect against diseases related to selenium deficiency [2,3]. Plants absorb selenium as selenite, selenate, and specific organic forms [4]. The studies on biofortification of edible plants with different selenium compounds (organic, especially synthetic, as well as inorganic forms) [5,6], revealed, that plants from *Brassicaceae* family had one of the highest capability to synthesize and accumulate selenium [6,7]. Additionally, brassica vegetables and functional foods products (sprouts of kale and kohlrabi) had shown chemopreventive properties, which could be additionally enhanced by fortification with different trace elements [6,8]. Selenium biofortification of brassica

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vegetables has been so far achieved by means of hydroponic cultures, soil fertilization and foliar spraying, resulting in varying amounts of selenium uptake [9]. However, as it is well known, selenium in higher concentrations may be toxic for plants [10], thus the adequate doses used during biofortification process are of crucial importance.

Most of the selenium compounds used so far for the biofortification of sprouts are inorganic selenium derivatives, while organo-selenium compounds have been scarcely examined, mainly because of their high instability [11]. On the other hand, organic selenium forms display interesting biological activity (i.e. anticancer, anti-inflammatory or even modulation of drug resistance of cancer cells) [12,13], thus there is a constant need for searching for new organic structures with improved stability. Various organo-selenium templates have been investigated previously in *in vitro* and *in vivo* studies such as methylseleninic acid, selenides, diselenides, selenocyanates, selenoesters, ethaselen, ebselen, selenium-containing 5-membered rings (selenophenes, selenazoles and selenadiazoles) or Se-containing 6-membered rings. Our recent study [14] on the biofortification of kale sprouts with 6 new synthesized selenium compounds (Se, Se-bis(cyanomethyl) benzene-1,3-bis (carboselenolate) (EDAG-11); Se-2-amino-2-oxoethyl benzoselenoate (EDA-117); Se-cyanomethyl 4-chlorobenzoselenoate (E-NS-4); Se-cyanomethyl 4-fluorobenzoselenoate (E-NS-17); Se-(2-oxopropyl) 4-chlorobenzoselenoate (EDA-71); 1-phenyl-4-(3-(phenylselenyl)propyl) piperazine (WA-4b)) (see Fig. A3) revealed significant differences between the doses of selenium delivered to sprouts by the above-mentioned compounds. A small percentage of supplemented selenium (<10%) was incorporated into kale sprouts as seleno-L-methionine, Se (IV) and Se-methylselenocysteine, while several other low molecular weight selenium compounds were also identified, in different proportions, depending on the compound used in the fortification process.

Following our previous findings regarding the influence of novel selenium organic compounds on the fortification of kale sprouts and the recognition of low molecular weight selenium metabolites in the sprouts, we decided to verify and compare the indirect biological effect exerted by the mentioned compounds. Thus, the present study was focused on the evaluation of the influence of these compounds on the synthesis of glucosinolates (GLS), isothiocyanates (ITC), indoles and phenolic acids in kale sprouts, together with the determination of their impact on antioxidant, anti-inflammatory and cytotoxic activity on gastrointestinal, prostate, and thyroid normal and cancer cells. Moreover, we tried to preselect the most effective compounds for the fortification process, with the application of advanced chemometric methods, revealing the relationship between total selenium, low molecular weight metabolites (obtained from previous work experiment, [14]) and phenolic and sulphur compounds crucial for biological activity of kale sprouts.

2. Material and methods

2.1. Reagents

LC/MS-grade acetonitrile and LC/MS-grade methanol were from Sigma-Aldrich (Seelze, Germany). Formic acid and ammonia for LC/MS was from Sigma-Aldrich. HPLC grade water was obtained from HLP 5 (HYDROLAB Poland) apparatus and was filtered through 0.2 μm filter before use. Chloramphenicol (≥98%, TLC) was from Sigma-Aldrich (Seelze, Germany). 3,3′-methanediylbis(1H-indole) (3,3′-diindolylmethane, DIM), 1H-indol-3-ylmethanol (indole-3-carbinol, I3C), indole-3-acetonitile, sulforaphane, allyl isothiocyanate, benzyl isothiocyanate, butyl isothiocyanate, phenyl isothiocyanate, phenethyl isothiocyanate, glucoiberin, progoitrin, glucoerucin and sinigrin standards were from Sigma-Aldrich (Seelze, Germany). Standards for HPLC analysis of phenolic acids: chlorogenic acid, iso - chlorogenic acid, sinapic acid, caffeic acid and protocatechuic acid were purchased from Fluka Chemie (Buchs, Switzerland). Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid); $FeCl_3 \cdot 6H_2O$; 1,1-diphenyl-2-picrylhydrazyl (DPPH), were from Sigma Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO), chloroform, HPLC grade acetonitrile, water and formic acid were purchased from Sigma-Aldrich (Seelze, Germany). 2,4,6-Trispyridyl-s-triazine (TPTZ) was purchased from Fluka Chemie (Buchs, Switzerland). Methanol, acetic acid, ammonium hydroxide solution, hydrochloric acid, sodium acetate and sodium carbonate were from Avantor Performance Materials Poland S.A. (Gliwice, Poland). All reagents were of analytical grade. Distilled water was purchased from Sigma-Aldrich.

2.2. Plant material

Kale seeds (*Brassica oleracea* L. var. *sabellica*) were collected from the plants harvested in 2019 in north-west Poland. Voucher specimen (No#BOS/PP/PL1045) was placed in the Department of Food Chemistry and Nutrition Jagiellonian University Medical College. The seeds were immersed for 3 h in water (50 mL of milliQ) without selenium (control) or with the organic selenium compounds, denoted as E-NS-4, E-NS-17, EDA-71, EDAG-11, EDA-117, WA-4b, JW-1, JW-2, JW-5 (selenium concentrations of 15 mg/L). Detailed chemical names and structures of used selenium compounds were presented in introduction part, appendixes (A2, A3) and in graphical abstract, respectively. The all-detailed information about the synthesis of compounds used was described by Zagrodzki et al. [14]. The seeds were transferred to a plastic sprouts' maker and grown for 7 days after seeding in the greenhouse of Garden of the Medicinal Plants, Faculty of Pharmacy Medical College, at 25 ± 2 °C, 70% of humidity, in sunlight exposure (10 h/day), being watered every day (150 mL).

2.3. Preparation of extracts

For sulphur compounds analysis, the fresh kale sprouts were thoroughly mashed in a mortar for over 1.5 min, and then incubated in a closed forced-air oven at 30–40 °C for 4 h to promote hydrolysis of GLS to ITC by myrosinase [15]. The obtained material was then extracted with methanol in a Soxhlet apparatus, as described previously [16]. The obtained extracts were decanted, centrifuged, stored in darkness in a freezer at − 20 °C and analysed after thawing for phenolic and sulphur compounds and antioxidant capacity. The methanol extracts were further evaporated, and dry residues were dissolved in DMSO for the evaluation of cytotoxic and anti-inflammatory activity.

2.4. Quantitative and semi-quantitative UPLC-MS/MS analysis of GLS, ITC and indole isothiocyanates

2.4.1. Preparation of samples

Each sample (100 μL) was mixed with 100 μL of 10 μg/mL chloramphenicol internal standard solution and brought to 1 mL with water making dilutions of the stock solutions of each sample. Each dilution was analysed in triplicate with UPLC-MS/MS.

2.4.2. Derivatization of samples

Each sample (100 μL) was mixed with 100 μL of 25% ammonia solution and the mixture was left in the room temperature overnight (ca. 16 h). Afterwards 55 μL of formic acid was slowly added to obtain pH in range 4–5. Finally, 100 μL of the 10 μg/mL chloramphenicol internal standard solution was added to the derivatized samples and diluted with water to 1 mL making derivatized dilutions of the stock solutions of each sample. Each derivatized dilution was analyzed in triplicate with UPLC-MS/MS. The content of the GLS: glucoerucin, glucoiberin, progoitrin and sinigrin was analyzed according to previously published method [19].

2.4.3. Apparatus

The UPLC-MS/MS system consisted of a Waters ACQUITY® UPLC® (Waters Corporation, Milford, MA, USA) coupled to a Waters TQD mass

spectrometer (electrospray ionization mode ESI-tandem quadrupole). Chromatographic separations were carried out using the Acquity UPLC BEH (bridged ethyl hybrid) C18 column, 2.1 × 100 mm, and 1.7 μm particle size. The column was maintained at 40 °C and eluted under following conditions: isocratic elution with 100% of eluent A over 1 min, afterwards linear gradient elution from 100% to 0% of eluent A over 8 min and 100% of eluent B over 1 min, at a flow rate of 0.3 mL/min. Eluent A: water/formic acid (0.1%, v/v); eluent B: acetonitrile/formic acid (0.1%, v/v). 10 μL of each sample were injected in triplicate.

Waters TQD mass spectrometer was calibrated for quantitative analysis using the solution of the investigated compounds at a 20 μg/mL concentration at a 20 μL/min flow and mixture of eluent A and B 1:1 (v/v) at a 0.28 mL/min flow. Optimized settings were as follows: source temperature 150 °C, desolvation temperature 350 °C, desolvation gas flow rate 600 L/h, capillary potential 3.00 kV, collision gas flow 0.1 mL/min. Cone potential and collision energy were individually optimized for each transition using the solution of the investigated compounds (Table A1). For ITC the solutions after derivatization to thiourea derivatives were used. Nitrogen was used for both - nebulizing and drying gas. Argon was used as collision gas. Traces of analyzed compounds were analyzed using MRM (Multiple Reaction Monitoring) method. All analytical data were processed using MassLynx V4.1 software (Waters Corporation, Milford, MA, USA). All analyses were performed in triplicate and the mean value was expressed in mg/100 g dry weight (dw).

2.5. HPLC determination of phenolic acids

The identification and quantification of phenolic acids in kale sprouts were performed as described previously [17] on Dionex HPLC system, equipped with PDA 100 UV–VIS detector and Hypersil Gold (C-18) column (5 μm, 250 × 4.6 mm, Thermo EC). Mobile phase consisted of 1% formic acid in water (A) and acetonitrile (B), 5–60% B in 60 min (flow rate 1 mL/min), with the detection wavelengths 254 and 285 nm. The compounds were identified by comparing their retention time with the reference standards. Quantitative analysis was carried out by measuring the peak area with regards to the appropriate standard curve (range of concentrations 0.0625–1 mg/mL). All analyses were performed in triplicate and the mean value was expressed in μg/g dw.

2.6. Determination of the antioxidant capacities

DPPH method: DPPH (3.9 mL, 25 mg/L) in methanol was mixed with the kale sprouts extracts (0.1 mL). The reaction was monitored at 515 nm until the absorbance was constant. Each sample was measured with three replicates. The mean capacity was expressed as mM Trolox/100 g dw.

FRAP method: The fresh working FRAP solution was prepared by mixing 2.5 mL 10 mM ferric-tripyridyltriazine with 40 mM HCl, 2.5 mL 20 mM FeCl3·H2O and 25 mL 0.3 mol/L acetate buffer, pH 3.6. FRAP reagent (900 μL) was mixed with 90 μL of distilled water and 30 μL of extract samples or the appropriate reagent blank and measured at 593 nm. Each sample was measured in three replicates. The mean capacity was expressed as μg Fe^{2+}/g dw.

Antioxidant activity was evaluated using a Biotek Synergy microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

2.7. Cytotoxic activity

Cytotoxic activity was tested on human cancer and normal cells, grouped as follows: prostate panel (prostate carcinoma DU-145, derived from metastatic site: brain, ATCC HTB-81; grade IV prostate carcinoma, PC-3, derived from metastatic site: bone, ATCC CRL-1435; prostate epithelial cells PNT2, ECACC 95012613), gastrointestinal panel (colorectal adenocarcinomas Caco-2, ATCC HTB-37, and HT-29, ATCC HTB-38; hepatocellular carcinoma HepG2, ATCC HB-8065), thyroid panel (follicular thyroid carcinoma FTC-133, ECACC 94060901; undifferentiated thyroid carcinoma 8505C, ECACC 94090184, thyroid follicular epithelial cells Nthy-ori 3–1, ECACC 90011609). Cells were grown at standard conditions (37 °C, 5% CO2, relative humidity) and culture media (DMEM/F12 for PNT2, HT29, HepG2, PC3, FTC133, 8505C; DMEM Low Glucose for DU145; MEM with NEAA for Caco2, RPMI1640 for Nthy-ori 3–1), supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics solution (10 000 U penicillin and 10 mg streptomycin/mL). The examined extracts were diluted in the culture media from freshly made stock solution in DMSO (10 mg/mL) to the working concentrations (from 0 to 500 μg/mL). Cell viability was determined by LDH assay, as described previously [18]. Briefly, cells were seeded onto 96-well plates (1.5×10^4 cells/well) and cultured for 24 h. Then, the culture medium was replaced with the same medium containing different concentrations of the tested extracts. After 24 of incubation, cell viability was determined. The absorbance was measured at 490 nm using a Biotek Synergy microplate reader (BioTek Instruments Inc., Winooski, VT, USA). All analyses were performed in triplicate, the results are expressed as % of cell viability (mean ± SD) and IC_{50} values (concentration at which the viability is inhibited by 50 percent).

2.8. Inflammation in vitro model

For anti-inflammatory assays RAW 264.7 murine macrophages were seeded onto 96 multi-well plates (1.5×10^5 cells/well) and pre-treated with the tested kale sprouts extract (50 and 100 μg/mL) for 1 h, followed by the addition of 10 ng/mL of LPS to induce inflammation process, according to Paśko et al. [19]. Dexamethasone (0.5 μg/mL) was used as a reference drug. The incubation was continued for the next 24 h. Cell culture supernatants were used for further analysis.

2.9. Nitric oxide determination

Griess Reagent Kit was obtained from Promega Corporation (Madison, Winooski, VT, USA), and the nitric oxide evaluation was performed according to the manufacturer's instructions. The analysis was performed in cell culture supernatants (see paragraph 2.8.) in three replicates, using a Biotek Synergy microplate reader (BioTek Instruments Inc., Winooski, VT, USA) and shown as % of LPS control.

2.10. TNF-alpha and IL-6 analysis

Cytokine determination was performed with Human ELISA kits (Bioassay Technology Laboratory, Shanghai, China) according to the manufacturer's instructions. Cell culture supernatants (see paragraph 2.8.) were used for the analysis, which was done in three replicates, using a microplate reader (BioTek Instruments Inc., Winooski, VT, USA) and shown as % of LPS control.

2.11. Statistical analysis

All experiments were performed in triplicate, and the data were reported as the mean ± standard deviation (SD). The results obtained for subsequent groups were analysed using one-way analysis of variance (ANOVA) followed by a post hoc Tukey's test. The differences among the groups were considered statistically significant when the p values were 0.05 or less. This part of statistical analyses was done using STATISTICA v. 13.3. (TIBCO Software Inc., Palo Alto, CA, USA).

The mixed hierarchical approach, including principal component analysis (PCA) and Partial Least Square (PLS) models, was used to reveal the correlation structure between the investigated parameters and to improve the interpretability of results [20,21]. Firstly, new variables were created by blocking original data (concerning selenium and its compounds' concentrations in the evaluated kale sprouts (see the comments in discussion), their water-soluble fractions and in proteolytic extracts [14] into meaningful subgroup, for which PCA model was built. New variables obtained as first two principal components (t1 and t2,

Table 1
Concentrations of sulphur compounds, phenolic acids, and antioxidant activities in kale sprouts treated with newly synthesized organic selenium compounds.

N = 3	EDAG-11	EDA-117	E-NS-4	E-NS-17	EDA-71	WA-4b	CONTROL
	Isothiocyanates (ITC) [mg/100 g dw]						
phenethyl isothiocyanate	0.10 ± 0.03***	0.13 ± 0.02***	0.10 ± 0.01***	0.11 ± 0.02***	0.11 ± 0.02***	152.8 ± 10.1***	0.20 ± 0.01
benzyl isothiocyanate	Nd	Nd	Nd	Nd	Nd	152.70 ± 4.13	Nd
butyl isothiocyanate	1.68 ± 0.18	1.90 ± 0.32***	2.36 ± 0.20***	2.62 ± 0.25***	1.52 ± 0.17	159.1 ± 10.6***	1.59 ± 0.02
phenyl isothiocyanate	Nd	Nd	3.31 ± 0.27	3.74 ± 0.48	2.12 ± 0.20	156.0 ± 8.9	Nd
allyl isothiocyanate	4.15 ± 0.59***	4.25 ± 0.78***	2.10 ± 0.14	3.77 ± 0.25***	6.14 ± 0.29***	3.25 ± 0.85	2.52 ± 0.13
Sulforaphane	7.91 ± 0.76***	8.31 ± 1.45***	7.10 ± 0.26***	11.8 ± 1.9***	12.1 ± 1.8***	8.08 ± 1.04***	3.76 ± 0.24
	Indole isothiocyanates [mg/100 g dw]						
indole-3-carbinol (I3C)	69.9 ± 1.4	Nd	135.9 ± 9.0***	129.8 ± 18.4***	56.3 ± 7.9*	68.6 ± 2.6	64.1 ± 2.2
indole-3-acetonitile (I3CN)	Nd	Nd	Nd	0.11 ± 0.02	Nd	Nd	Nd
3.3′diindolyl-methane (DIM)	7.02 ± 0.18	Nd	11.5 ± 1.4***	Nd	6.82 ± 0.35	4.74 ± 0.45***	7.07 ± 0.25
	Glucosinolates (GLS) [mg/100 g dw]						
Sinigrin	145.5 ± 12.3***	158.7 ± 27.3***	506.6 ± 22.1***	732.9 ± 24.3***	31.9 ± 1.7	194.1 ± 8.2***	37.6 ± 1.1
Progoitrin	24.6 ± 1.1***	27.2 ± 4.7***	30.7 ± 4.6***	133.7 ± 14.6***	6.75 ± 0.23*	34.4 ± 4.1***	4.56 ± 0.21
Glucoiberin	96.1 ± 5.6***	102.9 ± 17.3***	308.5 ± 26.3***	101.5 ± 7.8***	100.1 ± 6.8***	95.3 ± 4.3***	25.4 ± 1.6
Glucoerucin	0.77 ± 0.07	0.80 ± 0.13	1.33 ± 0.16**	1.52 ± 0.25***	0.67 ± 0.05*	0.80 ± 0.05	0.81 ± 0.02
	Phenolic acids [μg/g dw]						
Chlorogenic acid	0.47 ± 0.01**	0.48 ± 0.01 **	0.29 ± 0.01 *	0.34 ± 0.02 **	0.74 ± 0.04 **	0.42 ± 0.01 **	0.18 ± 0.02
Isochlorogenic acid	8.49 ± 0.62	10.1 ± 0.6**	17.1 ± 0.7 **	21.6 ± 0.1 **	7.32 ± 0.39	8.79 ± 0.09	6.74 ± 0.45
Proto-catechuic acid	0.15 ± 0.01*	0.26 ± 0.02**	0.40 ± 0.02***	0.45 ± 0.01***	0.14 ± 0.01*	0.20 ± 0.00**	0.10 ± 0.00
Caffeic acid	0.08 ± 0.00	0.17 ± 0.01**	0.28 ± 0.01***	0.34 ± 0.03 **	0.06 ± 0.01	0.02 ± 0.01 **	0.06 ± 0.00
Sinapic acid	0.66 ± 0.01**	0.37 ± 0.01	0.53 ± 0.01*	0.69 ± 0.01**	0.70 ± 0.02**	0.35 ± 0.01	0.40 ± 0.01
	Antioxidant activity						
FRAP [μg Fe^{2+}/g dw]	121.9 ± 0.9***	94.7 ± 2.6	148.6 ± 4.5***	187.4 ± 1.3***	83.3 ± 0.2***	78.4 ± 0.8***	91.0 ± 2.4
DPPH [mM Trolox/100 g dw]	32.6 ± 1.0	29.5 ± 1.0	46.5 ± 0.6**	62.8 ± 4.9***	27.4 ± 0.6	26.6 ± 1.0	28.5 ± 1.3

Values are presented as the mean ± SD (standard deviation) of three independent experiments in triplicate. Statistical analyses were carried out by using one-way ANOVA and Tukey post-hoc test with *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$ against the control kale sprouts.

respectively) for this subgroup were then used in superior (hierarchical) PLS model. The optimal number of latent components of PLS model was selected based on the following criteria: eigenvalue of respective component higher than 1, relatively high percentage of variance explained by the model (>50%); the convenience of making respective plot interpretation. PLS model correlated the predictive parameters (here: allyl isothiocyanate, caffeic acid, chlorogenic acid, glucoerucin, indol-3-carbinol, *iso*-chlorogenic acid, progoitrin, protocatechuic acid, sinigrin, sulforaphane, sinapic acid, t1 and t2) and response ones, that were in our study: FRAP, DPPH, TNF-alpha, IL6, DU-145, Caco-2, PC-3 and 8505C. The parameters with large weights (>0.3) in PLS model were assumed to be correlated. The parameters were considered negatively correlated if their weights within the PLS model showed the opposite signs; otherwise, they were considered positively correlated. To express the strength of bivariate associations, for the pairs of correlated parameters the algebraic products of their corresponding weights and cosine of corresponding angle were calculated (these coefficients are called the correlation weights). The "corresponding angle" means the angle determined by two lines connecting the origin with coordinates of both parameters on the PLS weights plot. PLS approach was also applied with a view to check possible similarities/dissimilarities in PLS score plot. The multidimensional statistical analysis was carried out using package SIMCA-P v.9 (Umetrics, Umeå, Sweden). The correlation weights were calculated using software delivered by MP System Co (Chrzanów, Poland). The package STATISTICA v. 13.3. was used for graphic representation of data.

3. Results and discussion

Searching for plants and functional foods fortified with selenium may be a valuable solution for reducing its deficiency in daily diet. Thus, in our investigation we focused on biofortification of kale sprouts with novel organic selenium compounds (JW-1, JW-2, JW-5, EDAG-11, EDA-117, E-NS-4, E-NS-17, EDA-71, WA-4b), recently synthesized and described in our previous work [14,22], which were applied to kale seeds in the concentration of 15 mg/L of selenium. What should be noted, three of the examined compounds (JW-1, JW-2, JW-5, Fig. A2) were toxic for the plants, which was observed as stunting of growth, wilting, and drying of sprouts, accompanied by premature death of the kale sprouts, hence these compounds were excluded from further investigation. Similar observation was made by Terry et al. [23] who also noticed root shortening, chlorosis, decreased protein synthesis, and indicated that selenium toxicity depends on the species and the environment. The selenide derivatives, such as JW-1, JW-2 and JW-5 bearing alkylphenylselenyl chain, contain additional imidazolidine-2,4-dione scaffold, which possibly makes them prone to induce cell death. In fact, it has been previously reported that molecules enclosing imidazolidine-2,4-dione scaffold exert cytotoxic activities [24,25]. In contrast, the remaining organoselenium compounds enclose benzoselenoate scaffolds (E-NS-4, E-NS-17, E-NS-71, EDA-11, EDA-117) or selenyl phenylpiperazine moiety (WA-4b), and are deprived of imidazolidine-2,4-dione ring (Fig. A3).

3.1. Influence of new organic selenium compounds on sulphur compounds concentration in kale sprouts

In the first part of the study, the fortification effect of the evaluated selenium compounds on the concentration of ITC (allyl isothiocyanate, benzyl isothiocyanate, butyl isothiocyanate, phenyl isothiocyanate, phenethyl isothiocyanate, and sulforaphane) was investigated. The most noticeable influence was observed in case of phenylselenyl derivative (WA-4b), which induced enormous increase in the concentration of benzyl isothiocyanate, butyl isothiocyanate, phenyl isothiocyanate, phenethyl isothiocyanate in kale sprouts, in comparison to the control (Table 1). Such a remarkably high concentration of butyl and aryl isothiocyanates can be attributed to the presence of a phenylalkylselenyl chain in WA-4b, a unique structural feature that distinguishes it from the remaining benzoselenoate derivatives (Fig. A3). Given the prominence of phenylalkylselenyl motif, we believe that molecular editing with selenium will have a strategic value in future functional food industry and agrochemistry. The sulforaphane concentration increased more than twofold (8.08 ± 1.04 vs. 3.76 ± 0.24 mg/100 g dw.) in WA-4b fortified sprouts, and similar increase was also observed in the sprouts fortified with the remaining selenium compounds. Interestingly, the other selenium organic compounds (i.e. EDAG-11, EDA-117, E-NS-17, and EDA-71) influenced significantly the production of allyl isothiocyanate, as

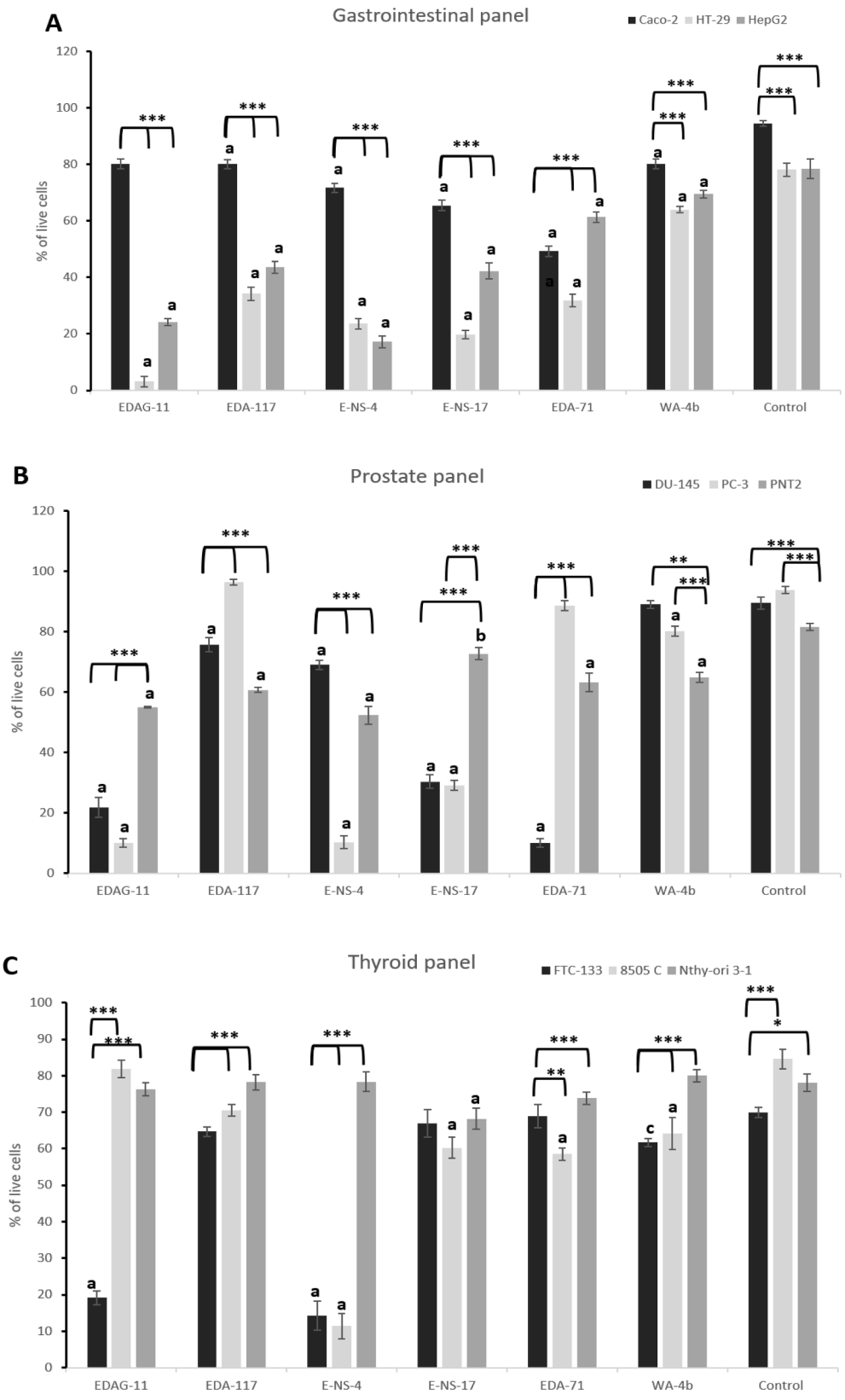


Fig. 1. The cytotoxic effect of control and biofortificated with new selenium compounds (EDAG-11; EDA-117; E-NS-4; E-NS-17; EDA-71; WA-4b) kale sprouts on gastrointestinal (A), prostate (B) and thyroid (C) cells. Cells were treated with 500 μg/mL of sprouts extracts for 24 h. Values are presented as the mean ± SD (standard deviation). Statistical analyses were carried out by using one-way ANOVA and Tukey post-hoc test. Significant differences (*: $p < 0.05$, **: $p < 0.01$, and *** $p < 0.001$) between different cell lines for the same extract were marked by upper black line. Significant differences between the same cell line treated with selenium compounds versus control sprouts were marked as a: $p < 0.001$, b: $p < 0.01$; c: $p < 0.05$.

we observed significant increase in allyl isothiocyanate concentration in comparison to control kale sprouts (Table 1).

In case of indole isothiocyanates (DIM; I3C; and indole-3-acetonitrile) significant increase in concentration of I3C was observed for kale sprouts fortified with E-NS-17 and E-NS-4, while the latter compound also caused an increase in the concentration of DIM in the tested sprouts. For the remaining selenium compound no influence or significant decrease (EDA-71 for I3C and WA-4b for DIM) was observed, which is not surprising in the light of the study of Piekarska et al. [26], who found that only 1% of GLS was converted into indoles in selenium

Table 2
IC_{50} of the selenium fortified with new organic selenium compounds and control kale sprouts.

IC_{50} μg/mL	EDAG-11	EDA-117	E-NS-4	E-NS-17	EDA-71	WA-4b	CONTROL
	Gastrointestinal panel						
Caco-2	>Cmax	>Cmax	>Cmax	>Cmax	479.6	>Cmax	>Cmax
HT-29	163.2	231.1	242.8	269.6	337.6	>Cmax	>Cmax
HepG2	207.7	374.7	101.9	324.1	>Cmax	>Cmax	>Cmax
	Prostate panel						
DU-145	318.8	>Cmax	>Cmax	193.8	98.8	>Cmax	>Cmax
PC-3	79.7	>Cmax	78.5	226.5	>Cmax	>Cmax	>Cmax
PNT-2	>Cmax	>Cmax	>Cmax	>Cmax	>Cmax	>Cmax	>Cmax
	Thyroid panel						
FTC-133	177.6	>Cmax	79.9	>Cmax	>Cmax	>Cmax	>Cmax
8505C	>Cmax	>Cmax	80.1	>Cmax	>Cmax	>Cmax	>Cmax
Nthy-ori 3–1	>Cmax	>Cmax	>Cmax	>Cmax	>Cmax	>Cmax	>Cmax

fortified different brassica sprouts.

Our investigation on new organic selenium compounds indicates that most of them can significantly increase the amount of evaluated GLS (sinigrin especially by E-NS-17 and E-NS-4, progoitrin especially by E-NS-17, glucoiberin especially by E-NS-4) with the lowest influence on the level of glucoerucin, whose synthesis was stimulated significantly only by the fortification of E-NS-17 and E-NS-4 (Table 1). What is worth to underline, the level of GLS in the sprouts after fortification with selenium has become comparable to their content in leafy and root brassica vegetables. It was observed particularly in case of E-NS-17 compound, which caused an increase in GLS content in the sprouts approaching that in mustard green leaves (for sinigrin), in green cauliflower (for glucoiberin), and in turnip and Chinese cabbage (for progoitrin) [27]. Such high amount of GLS in the evaluated kale sprouts, even after hydrolysis, is in agreement with results of Finley et al. [28], who noted that high concentration of selenium may suppress GLS breakdown in plants. The concentration of GLS in nonfortified kale sprouts is in general agreement with Verkerk et al. [27] and Šamec et al. [29]. The increase in GLS content, observed in our study, is in opposition to the most of the so-far published results on brassica sprouts (broccoli, mustard, cabbage) fortified with well-known selenium compounds like SeO_2 (10 mg/L), selenite (Na_2SeO_3, 100 μmol/L) and selenate (Na_2SeO_4, 100 μmol/L), where no effect on the total GLS content was observed [26,30]. Some other studies reported that total GLS content is minimally affected by selenate treatment in broccoli sprouts and mature broccoli [7,31]. Tian et al. [30] noticed that the effect of selenium fortification depends on broccoli cultivar used in the study. In case of FL60 and SL120 cultivars total GLS content in the sprouts was not affected after Se fertilization, while an increase by 15.2% after selenate treatment was noted for the cultivar WX90. This observation was also confirmed by Gu et al. [32] who observed that the GLS structure and content in plants are easily affected by plant species, genotypes, and plant growth conditions (light, herbivory, pathogen infection). Moreover, the resemblance between Se and S implies that these elements share common metabolic pathways in plants. Tian et al. [33] indicated that the levels of the GLS precursors methionine and phenylalanine, as well as the expression of genes involved in GLS biosynthesis, were greatly decreased by selenium (Na_2SeO_4) supplementation. The discrepancy in our results and those obtained by other authors may be also explained by the use of different selenium species, as most of the published data concerns the sprouts exposed to different forms of inorganic selenium [7,34–35], while no data exists on selenium organic compounds used for biofortification. Not only Se species but also the method of supplementation may play a crucial role for the success of biofortification [36]. Abdalla et al. [37] found that the enrichment of the selenium concentration by fertilization through the foliar application can significantly affect the synthesis of GLS hydrolysis products and enhance the quality and health benefits of pak choi (*Brassica rapa L. var. chinensis*).

3.2. Influence of new organic selenium compounds on phenolic acids concentration

Apart from sulphur compounds, we also evaluated the changes of phenolic acids (chlorogenic acid, isochlorogenic acid, sinapic acid, caffeic acid and protocatechuic acid) levels in selenium fortified sprouts. All used selenium compounds caused significant increase in the concentration of chlorogenic and protocatechuic acids (Table 1). The amount of sinapic acid increased significantly after fortification with EDA-71, E-NS-4, E-NS-17, EDAG-11, and EDA-117. Caffeic and isochlorogenic acids synthesis was stimulated by E-NS-17, E-NS-4, EDA-117 and by E-NS-17, EDA-117, respectively. Our results are in agreement with those described by other authors, on the positive effect of inorganic selenium fortification on the phenolic compounds content in brassica vegetables, like broccoli and turnip sprouts and leaves and radish roots [38–40]. Zagrodzki et al. [6] noticed that selenium (added as inorganic salts) differentially affected the production of phenolic acids, depending on the brassica sprouts species (kale and kohlrabi) and selenium dose, but generally found an increase in the content of these acids. Thiruvengadam and Chung [40] suggested that selenium-induced anthocyanins accumulation by the enhanced expression of genes related to their biosynthesis. The significant influence of SeO_2 ingestion on the expression of phenylalanine ammonia-lyase a key enzyme in the phenylpropanoid pathway and chalcone-flavanone isomerase which participates in the early step of flavonoid biosynthesis was noticed in turnip [40].

3.3. Influence of new selenium compounds on antioxidant activity of fortified kale sprouts

In next step of the experiment, we evaluated the impact of the selenium compounds on the antioxidant activity of the fortified sprouts. The tested compounds showed different effects, with E-NS-17, E-NS-4, and EDAG-11 causing the highest increase in antioxidant activity in fortified kale sprouts, in comparison to control group. Interestingly, WA-4b and EDA-71 compounds caused a slight, but significant decrease of the activity (Table 1). Our results indicate also that antioxidant activity of the tested sprouts, evaluated by both methods (FRAP and DPPH), was positively correlated with such bioactive compounds like isochlorogenic acid, sinigrin, glucoerucin, caffeic acid, protocatechuic acid and finally I3C (Table 3). The increased total antioxidant activity was reported in several Se-enriched brassica plants (broccoli florets, garden cress sprouts) [35,41]. In case of brassica sprouts, Piekarska et al. [26] found that antioxidant activity determined by DPPH for SeO_2 enriched mustard, broccoli and cabbage sprouts appeared higher, but such effect was not confirmed in case of ABTS test for the cabbage and broccoli sprouts. Steady increase in the antioxidant activity (FRAP, DPPH) with the increasing Se doses for kale sprouts was noted in our previous study, but no such trend, or even lower results for intermediate Se doses, were

Table 3
Correlation weights for the pairs of parameters based on PLS model (only first thirty correlation weights with highest absolute values were shown).

Pairs of correlated parameters		Correlation weights
Phenolic acids		
isochlorogenic acid	protocatechuic acid	0.129
protocatechuic acid	caffeic acid	0.124
Sulphur compounds		
allyl isothiocyanate	sulforaphane	0.203
sinigrin	glucoerucin	0.135
I3C	sinigrin	0.115
I3C	glucoerucin	0.109
Phenolic acids and sulphur compounds		
sinapic acid	sulforaphane	0.247
chlorogenic acid	sulforaphane	0.232
chlorogenic acid	allyl isothiocyanate	0.222
sinapic acid	allyl isothiocyanate	0.215
chlorogenic acid	sinapic acid	0.247
isochlorogenic acid	sinigrin	0.137
isochlorogenic acid	glucoerucin	0.135
caffeic acid	sinigrin	0.132
caffeic acid	glucoerucin	0.130
protocatechuic acid	sinigrin	0.129
protocatechuic acid	glucoerucin	0.127
isochlorogenic acid	I3C	0.119
caffeic acid	I3C	0.109
protocatechuic acid	I3C	0.103
FRAP, DPPH and antioxidants		
isochlorogenic acid	DPPH	0.144
FRAP	DPPH	0.143
sinigrin	DPPH	0.141
isochlorogenic acid	FRAP	0.139
glucoerucin	DPPH	0.138
sinigrin	FRAP	0.137
caffeic acid	DPPH	0.135
glucoerucin	FRAP	0.133
caffeic acid	FRAP	0.131
protocatechuic acid	DPPH	0.131
protocatechuic acid	FRAP	0.127
I3C	DPPH	0.124
I3C	FRAP	0.121
Cell lines and antioxidants		
DU-145	Caco-2	0.255
chlorogenic acid	DU-145	−0.253
chlorogenic acid	Caco-2	−0.221
allyl isothiocyanate	DU-145	−0.221
sinapic acid	DU-145	−0.214
sulforaphane	DU-145	−0.210
allyl isothiocyanate	Caco-2	−0.193
sinapic acid	Caco-2	−0.178
sulforaphane	Caco-2	−0.178

observed for kohlrabi sprouts [6]. It suggests that kale sprouts are more sensitive to Se-stimulation, in comparison to kohlrabi sprouts. Such relation may be interpreted as greater ability of kohlrabi sprouts to equilibrate changing Se status within homeostatic process.

3.4. Influence of new selenium compounds on cytotoxic activity of fortified kale sprouts

Next, we decided to evaluate cytotoxic activity of the examined selenium-fortified kale sprouts on a number of cancer cells of different origin. We have chosen gastrointestinal, prostate and thyroid cancer cells, to determine the chemopreventive potential of the tested sprouts. To verify the safety and selectivity of the tested sprouts, the effect on appropriate normal cells was also tested. All tested kale sprouts extracts varied in their influence on the examined cell lines, in a dose-dependent manner (0.05–0.5 mg/mL). On Fig. 1ABC cytotoxic activity against cancer (Caco-2, HT-29, DU-145, PC-3, FTC-133, 8505C) and normal cells (HepG2, PNT2, Nthy-ori 3–1) was presented for the highest tested concentration (0.5 mg/mL). Additionally, what should be noted, control kale sprouts extracts were not toxic for normal cells used in this study (HepG2, PNT2 and Nthy-ori 3–1) in the tested concentration range (Fig. 1) and IC_{50} were under Cmax (Table 2).

3.4.1. Gastrointestinal panel

In case of evaluated gastrointestinal cells (Fig. 1A), Caco-2 seems to be the most resistant for the effect of the selenium compounds, with IC_{50} exceeding the maximal concentration used, excluding the sprouts fortified with EDA-71 compound (IC_{50} 480 μg/mL). This result is comparable to Luang-In et al. [42] for Se-enriched Chinese kale sprouts, with IC_{50} 346 μg/mL. All the tested selenium-fortified sprouts were toxic for HT-29 cells (IC_{50} from 163.2 μg/mL for EDAG-11 to 337.6 μg/mL for EDA-71) with one exception (WA-4b). Piekarska et al. [26] described no cytotoxic effect of selenium-enriched cabbage sprouts to HT29 cells. In case of HepG2 significant toxicity was observed for EDAG-11, EDA-117, E-NS-17, E-NS-4, with the lowest IC_{50} value (101.9 μg/mL) for the latter, which suggests low selectivity of the tested extracts but only moderate hepatotoxicity. Similar effect was obtained by Luang-In et al. [42] for Se-enriched Chinese kale sprouts, with IC_{50} 178 μg/mL.

3.4.2. Prostate panel

All evaluated selenium enriched sprouts were toxic for both prostate cancer cell lines, however, with lower toxic effect to normal prostate cells (Fig. 1B). The lowest IC_{50} for DU-145 was noted for EDA-71 (98,75 μg/mL) and for PC-3 for E-NS-4 (78.5 μg/mL) and EDAG-11 (79.7 μg/mL). What is most important, in case of the sprouts enriched with EDAG-11 and E-NS-4 compounds, strong and significant effect was noted for highly metastatic PC3 cells, in comparison to low metastatic DU-145 cells, with good selectivity against normal prostate cells. Additionally, this positive effect may be the result of stimulation of synthesis of chlorogenic acid, allyl ITC, sulforaphane and sinapic acid by the mentioned selenium compounds, which was confirmed by negative correlation between these active compounds and DU-145 cell line viability (Table 3). E-NS-4 is the example of selenoester (more precisely alkyl selenoesters), which may undergo reduction in the cells, resulting in the generation of redox active molecules involved in cell death, as it was observed especially against prostate cancer cells [43,44]. The Se-methylcyanates group occurs in the structure of both E-NS-4 and EDAG-11 compounds. On the other hand, it is known that selenocyanates showed effectiveness in the prevention and treatment of different prostate or colon cancers both in vitro and *in vivo* models [45–47]. Moreover, selenocyanates revealed no systematic toxicity and negative effect on kidney, liver, or heart, with the decreasing effect on melanoma growth [11]. The presence of cyanate group may be responsible for redox cycle, involving GSH, antioxidant enzymes (CAT, SOD, glutathione – S – transferase, TrxR, GPX) and promote apoptosis by induction of p53, BAX and inhibition of Bcl-2 [46,48–49]. All these effects, described for selenoesters, provide solid justification for further, in-depth studies of the selenoesters-fortified sprouts extracts. However, we are also aware that the final metabolites, which are active in extracts, can be completely different from the original compounds, as it was proven in our recent paper [14].

3.4.3. Thyroid panel

Sprouts enriched with two selenium compounds (E-NS-4, and EDAG-11) presented the most interesting cytotoxic activity against thyroid cancer cells (Fig. 1C). The highest cytotoxic effect was observed in case of E-NS-4 enriched sprouts, and the activity was comparable against both FTC-133 and 8505C (IC_{50} 80 and 80.14 μg/mL) cell lines, while the compound was not toxic to normal epithelial thyroid cells. The EDAG-11 enriched sprouts were cytotoxic to follicular carcinoma FTC-133 cells (IC_{50} 178 μg/mL), while the other, undifferentiated thyroid carcinoma 8505C cells, as well as normal thyroid cells, remained unaffected. According to our knowledge, it is the first study evaluating the effect of organic selenium compounds on the thyroid function in vitro. This

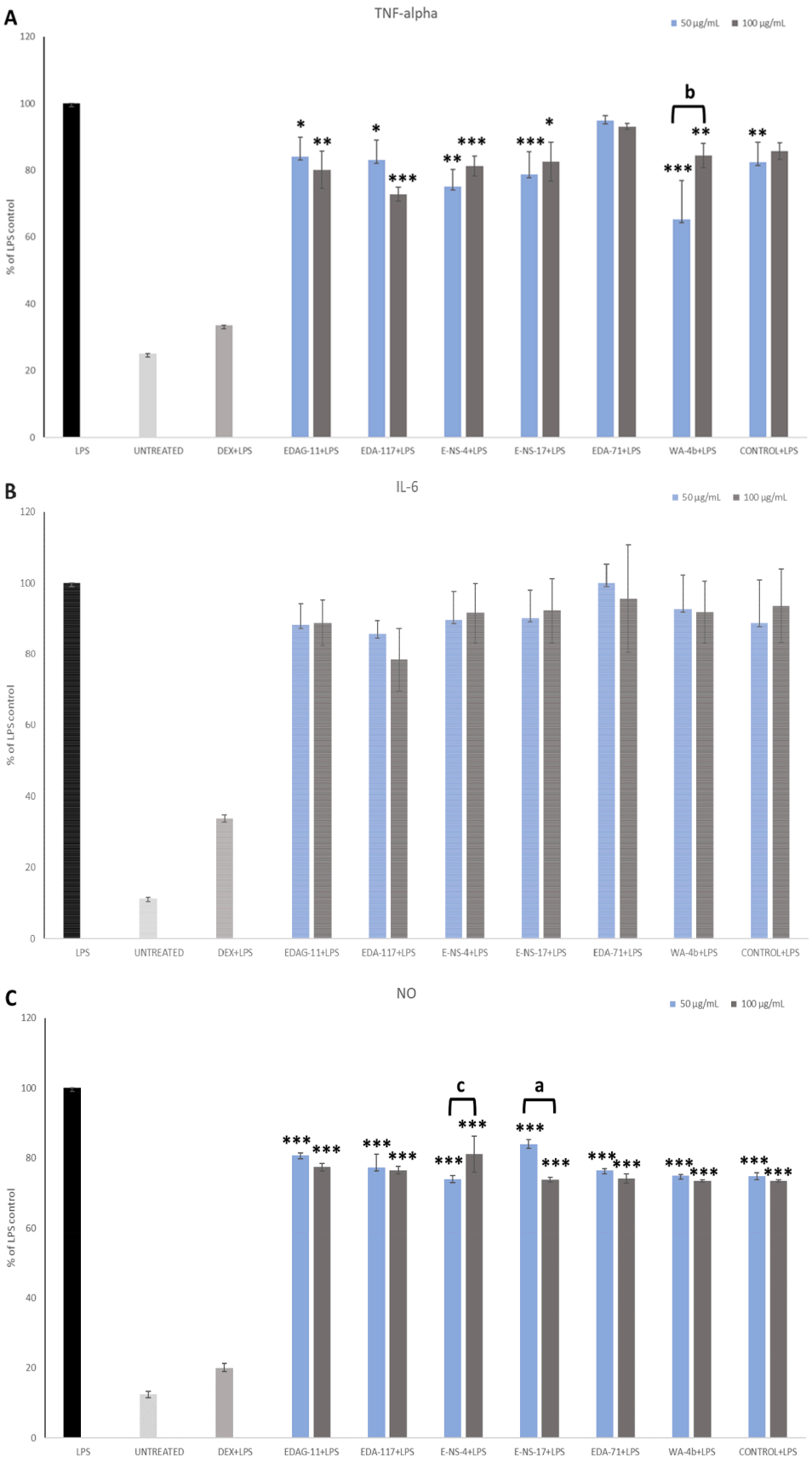
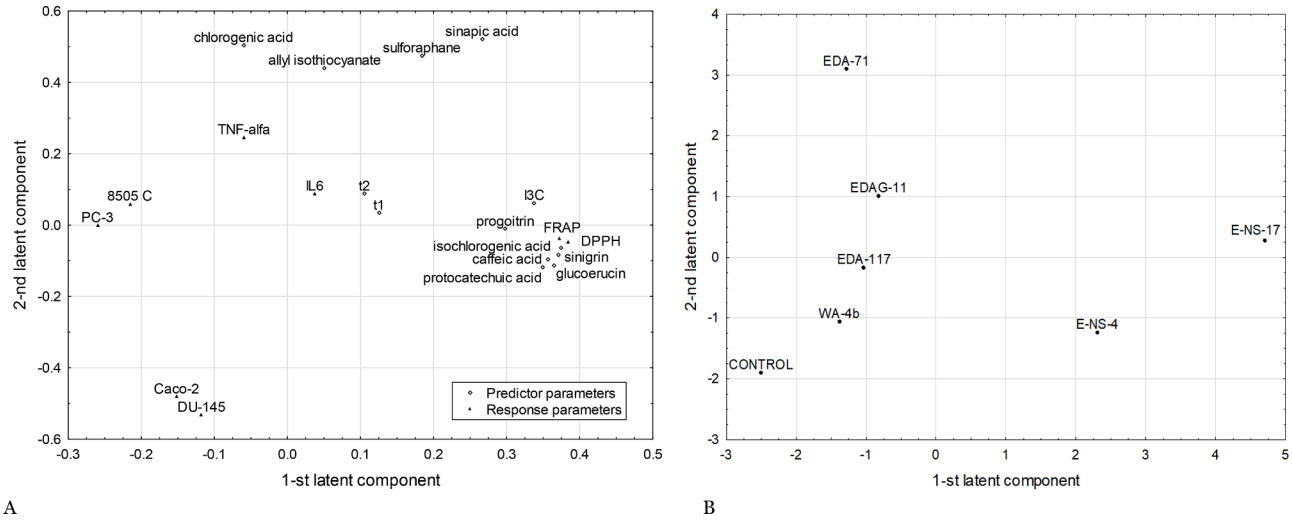


Fig. 2. The effect of control and biofortificated with new selenium compounds (EDAG-11; EDA-117; E-NS-4; E-NS-17; EDA-71; WA-4b) kale sprouts on: (A) tumour necrosis factor-alpha (TNF-alpha), (B) interleukin-6 (IL-6) and (C) nitric oxide (NO) release in LPS-stimulated RAW 264.7 macrophages. RAW cells were pre-treated with 50 and 100 µg/mL of sprouts extracts for 1 h, afterwards cells were incubated with (10 ng/mL) or without LPS (untreated) for the next 24 h. Dexamethasone (DEX) was used as a reference. Values are presented as the mean ± SD (standard deviation) of three independent experiments in triplicate. Statistical analyses were carried out by using one-way ANOVA and Tukey post-hoc test with *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$ against the LPS-stimulated cells. Significant differences between the doses were marked by upper black line (a: $p < 0.001$, b: $p < 0.01$; c: $p < 0.05$).



A B

Fig. 3. Parameters' weights (A), and the distribution of studied selenium fortified, and control kale sprouts in the space determined by the first two latent components (B).

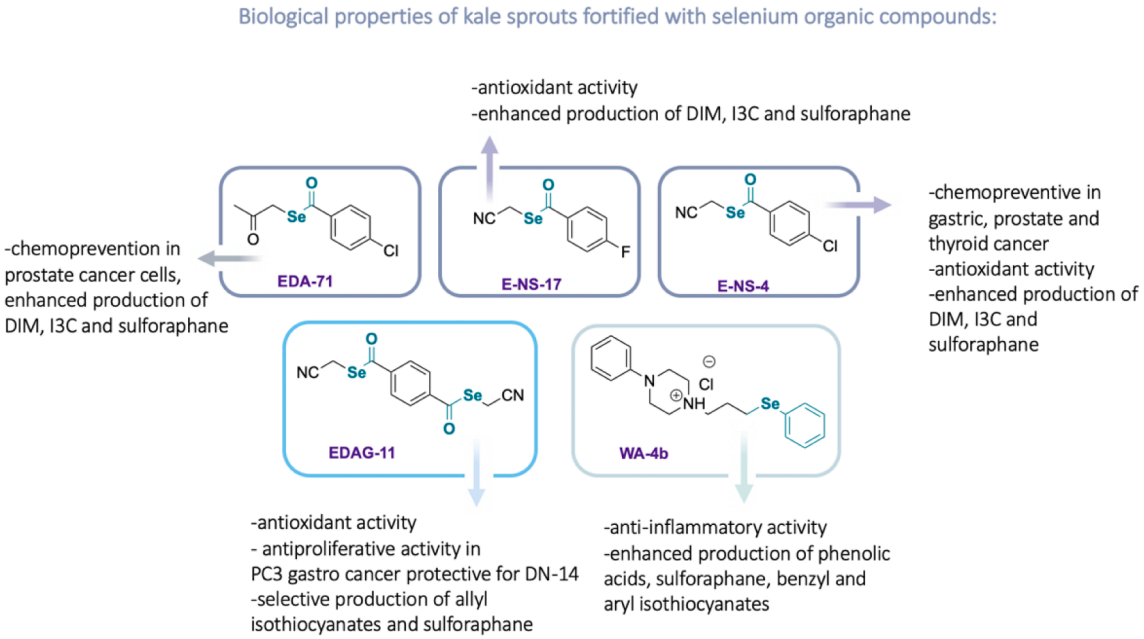


Fig. 4. Summary of the main findings regarding structure-biological activity relationship.

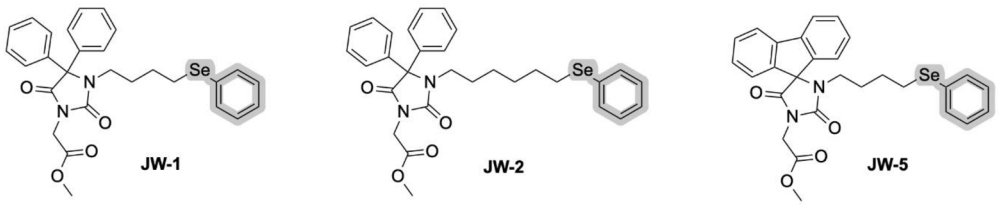


Fig. A2. Newly synthesized selenium organic compounds excluded from the further steps of kale sprouts fortification experiment.

observation is very important, as not only selenium, but also brassica vegetables may be involved in the homeostasis of thyroid glands [50,51]. Searching for new candidates for prevention of thyroid cancer, even in the form of functional foods, seems to be crucial, in the view of the statement by Rahib et al. [52] that thyroid cancer will replace colorectal cancer as the fourth leading cancer diagnosis by 2030.

3.5. Influence of kale sprouts fortified with selenium compounds on inflammation process

Inflammation seems to be one of the most important elements of carcinogenesis process [53], enabling cancer progression and decreasing the response to therapy. Searching for new candidates for functional

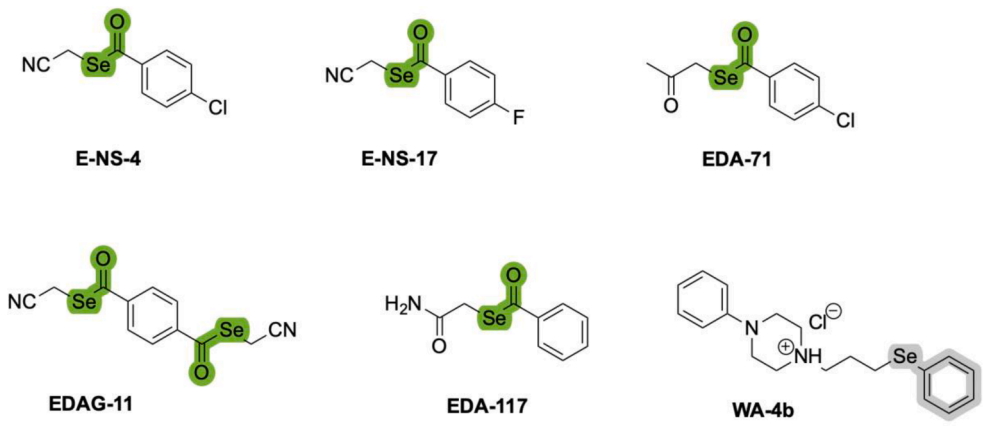


Fig. A3. Chemical structures of investigated selenium compounds.

Table A1
Optimized settings for quantitative analysis of the investigated sulphur compounds.

Compound	Mode	Rt [min]	Transition	Cone potential [V]	Collision energy [eV]
DIM	ES-	8.05	245.02 → 115.85*	46	24
I3C**	ES+	7.28	129.90 → 76.86*	48	28
			129.90 → 102.86		18
indole-3-acetonitrile	ES+	5.59	157.04 → 129.92*	10	10
			157.04 → 116.79		28
sulforaphane	ES+	4.48	178.00 → 113.90*	18	14
			178.00 → 71.79		26
allyl isothiocyanate	ES+	3.06	116.94 → 57.89*	16	10
			116.94 → 99.84		12
benzyl isothiocyanate	ES+	4.61	166.96 → 90.92*	30	24
			166.96 → 42.94		26
butyl isothiocyanate	ES+	4.17	132.91 → 56.92*	30	18
			132.91 → 73.96		12
phenyl isothiocyanate	ES+	3.96	152.95 → 93.92*	28	18
			152.95 → 135.87		22
phenylethyl isothiocyanate	ES+	5.03	181.10 → 104.92*	20	26
			181.10 → 76.91		36
chloramphenicol	ES-	4.94	320.98 → 151.73*	26	30

*Trace used for quantitation.
** [M-H2O] + used as parent ion.

foods with chemopreventive properties should include not only direct cytotoxic activity, but also the effect on cancer co-existing symptoms, like inflammation. Thus, as the final step of our investigation, we decided to verify the influence of the selenium-enriched kale sprouts extracts (in two doses of 50 and 100 μg/mL) on the release of the selected pro-inflammatory mediators (NO, IL-6, TNF-alpha) in LPS-stimulated RAW 264.7 macrophages). The results are presented in Fig. 2. A significant inhibition of NO synthesis was observed in macrophages pre-treated with all evaluated extracts, when compared to LPS-stimulated cells ($p < 0.001$). However, in most cases the effect was not dose-dependent, with two exceptions: E-NS-4 enriched sprouts were more effective at lower dose while for E-NS-17 enriched sprouts the higher dose stimulated higher anti-inflammatory response than the lower dose. In case of TNF-alpha, the evaluated sprout extracts significantly decreased its release when compared to LPS-stimulated cells, with the exception of EDA-71 compound. It was also noted that dose-dependent changes were observed only in case of WA-4b, where lower dose was more effective. For this structure (selenide) antioxidant potential associated with decreased inflammation process was confirmed [11]. In our study, also the slight suppression of IL-6 release was noted in LPS-stimulated macrophages pre-treated with kale sprouts fortified with selenium compounds, but the effect was not significant when compared to LPS-stimulated cells, and no dose-dependency was noted. What is worth noting, anti-inflammatory effect of Se-fortified brassica sprouts was described for the first time.

The anti-inflammatory effect of amaranth sprouts fortified with inorganic selenium compounds was reported [54], with the expression of cytosolic p65 subunit of NFκB in unstimulated RAW 264. The amount of p65 NFκB protein in cells was higher, but no influence on the process of NFκB translocation was observed. Recently, Se-containing structures (Se-NSAID hybrids and SeCN-aspirin analog) were introduced as the new idea of anti-cancer and anti-inflammatory molecules. The compounds showed selectivity against cancer cells, cell cycle arrest in G1 and G2/M phases, inducement of apoptosis and also inhibition of COX-2 [55]. These results may suggest that the evaluated seleno-alkyl-cyanates (E-NS-4, E-NS-17, EDAG-11), representing structures similar to the selenocyanates, should be further evaluated in this direction.

3.6. Chemometric assay

In this paper we evaluated influence of six new organic selenium compounds on the synthesis of active molecules and on biological activity of the fortified kale sprouts. To better explained the observed effects of those newly synthesized compounds on the above-mentioned activities, we decided to additionally include the results published in our recent study [14], together with the data presented in this paper, in one chemometric analysis.

The block of selenium and its compounds concentrations in the evaluated kale sprouts contained following parameters: total selenium concentration in sprouts (TSe), selenium concentration in proteolytic extracts (SePE), the concentrations of selenomethionine (SeMet), Se(IV) and Se-methylselenocysteine (SeMetSeCys) in sprouts - expressed as

percentage of concentration of selenium contained in these species (SeMet%TSe, Se(IV)%TSe, SeMetSeCys%TSe, respectively), the concentrations of selenomethionine, Se(IV) and Se-methylselenocysteine in proteolytic extracts of sprouts - expressed as concentration of selenium contained in these species (SeMet%SePE, Se(IV)%SePE, and SeMetSeCys %SePE, respectively). The PCA model, constructed for this set of parameters, had 2 significant components, with eigenvalues of 3.62 and 2.80, and explained 91.8% of variance of original parameters. The first principal component in this model (t1) had positive weights predominantly for the TSe and SePE, and negative for SeMetSeCys%TSe and SeMet%SePE (within each pair of parameters there were significant correlations). The second principal component (t2) was loaded mainly positively by SeMetSeCys%SePE, Se(IV)%SePE and Se(IV)%TSe. All these parameters, being in one tight cluster were strongly correlated with each other. The parameter SeMet%TSe deviated from this cluster and loaded negatively on second principal component.

The PLS model fulfilling leave-one-out cross-validation criteria was constructed for parameters listed in Statistical approach section. Other parameters were not included in the model as they were considered noninformative. The model had two significant components, and explained 74.1% of variance in the predictive parameters, and 56.7% of variance in the response parameters, with eigenvalues of 3.54 and 1.64, respectively. The loadings for first two latent components are shown on Fig. 3A.

The first latent component in this model had positive weights predominantly for some polyphenols and sulphur compounds (predictive parameters), as well as for DPPH and FRAP (response parameters). Therefore, the above-mentioned compounds had high correlation weights with both indices of antioxidant status. In particular, the highest positive correlation weights based on this latent component were revealed between isochlorogenic acid, DPPH, FRAP and sinigrin (Table 3). Negative weights on this component were below absolute value of 0.3. The parameters loading mainly positively on second component were in one cluster (sinapic acid, sulforaphane, chlorogenic acid and allyl isothiocyanate) and they had high correlation weights among themselves. All of them correlated negatively with cell lines Caco-2 and DU-145 (Table 3). Neither TNF-alpha and IL-6 nor t1 and t2 correlated significantly with other parameters. The whole set of significant correlation weights, grouped according to different categories of parameters, was shown in Table 3. The projection of selenium compounds into the space defined by first two latent components of PLS model was shown on Fig. 3B.

Visual inspection of score scatterplot of PLS model (Fig. 3B), which shows original selenium compounds in the space determined by first two latent components, disclosed one cluster containing three compounds (EDAG-11, EDA-117, WA-4b) and three other "isolated" compounds, with apparently distant biochemical properties. Two of them (E-NS-17 and E-NS-4) differed from the above-mentioned cluster mainly in terms of their influence on the concentrations of secondary metabolites in the sprouts (i.e., polyphenols and sulphur compounds: caffeic acid, glucoerucin, I3C, isochlorogenic acid, progoitrin, protocatechuic acid, sinigrin) as well as DPPH and FRAP values. One remaining compound (EDA-71) stimulated largely other, rather separated set of polyphenols and sulphur compounds (allyl isothiocyanate, chlorogenic acid, sulforaphane, and sinapic acid). It also caused a weaker response from the cell lines Caco-2 and DU-145 (cf. Fig. 3A and 3B).

4. Conclusions

Plants from *Brassicaceae* family biofortified with selenium may produce Se-containing compounds and GLS, which are precursors of cancer preventing substances. The determination of the biological effects of such new metabolites on the functioning of living organisms may be an interesting challenge for future studies. We have demonstrated that the germination of kale seeds watered with new organic selenium compounds (15 mg/L) is an efficient way of obtaining sprouts enriched with selected sulphur and phenolic compounds, without visible impact on sprouts physiology. In about 190 μmol/L concentration of new selenium molecules, GLS synthesis and profile of their breakdown products were significantly affected. The present study highlights the benefit of phenylalkylselenyl derivatives in the enhanced production of isothiocyanate compounds in kale sprouts (Fig. 4). The overall efficacy of a selenide derivative WA-4b indicates the interesting potential of the investigated scaffold, warrants its further examination in the field of agrochemistry. According to the cytotoxic activity, two directions for future, more detailed studies were revealed: EDAG-11 and EDA-71 as agents against prostate cancer cells and E-NS-4 against thyroid, prostate, and gastrointestinal cancers. Most of all selenium compounds decreased NO and TNF-alpha production in comparison to LPS stimulation of RAW cells but WA-4b was by far the most promising candidate for further anti-inflammatory studies. Although there were no strong positive correlations between selenium and selenium derivatives concentrations in the sprouts, and the synthesis of bioactive compounds or evaluated biological activity, the examined new selenium compounds caused indirect significant positive impact on the investigated processes and activities.

The observed neutral effect of non-fortified kale sprouts on normal human cells confirmed that these sprouts may be recommended as a good and safe element of the human diet. It should be highlighted that no extra sulphur addition was performed in our study and future studies should be focused on the relationship between Se and S during GLS metabolism in kale sprouts fortified with these new selenium compounds. Given that currently the chemical repertoire of potential fortifiers bearing selenium moieties is largely limited, the present study can facilitate the design of future agrochemicals. Compounds bearing benzoselenoate scaffold or selenyl phenylpiperazine motif seem to be particularly promising for these purposes.

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CRediT authorship contribution statement

Paweł Paśko: Conceptualization, Methodology, Project administration, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Agnieszka Galanty:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Paweł Zagrodzki:** Conceptualization, Methodology, Project administration, Formal analysis, Resources, Validation, Visualization. **Paweł Żmudzki:** Investigation, Writing – review & editing, Validation, Supervision. **Urszula Bieniek:** Data curation, Investigation. **Ewelina Prochownik:** Data curation, Investigation. **Enrique Domínguez-Álvarez:** Resources, Writing – review & editing. **Katarzyna Bierła:** Methodology, Formal analysis, Validation. **Ryszard Łobiński:** Validation, Supervision. **Joanna Szpunar:** Conceptualization, Resources. **Jadwiga Handzlik:** Methodology, Project administration, Resources, Supervision. **Monika Marcinkowska:** Writing – review & editing, Visualization. **Shela Gorinstein:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

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